Influence of Subinhibitory Concentrations of Clindamycin on Opsonophagocytosis of *Staphylococcus aureus*, a Protein-A-Dependent Process

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Incubation in subinhibitory concentrations of clindamycin enhanced the uptake of protein A-rich *Staphylococcus aureus* strains by polymorphonuclear leukocytes. This enhancement of uptake appeared to be due, in part, to a reduction in the amount of protein A at the bacterial cell surface by clindamycin.

Exposure to antibiotics at concentrations lower than the MIC is known to induce morphological changes in certain bacteria (5, 8), and it also renders these organisms more susceptible to phagocytosis (2, 4, 6–9). Because opsonization of *Staphylococcus aureus* is necessary for phagocytosis, the enhanced uptake by phagocytes of these antibiotic-treated bacteria may be due to more efficient opsonization (4, 10).

The synthesis of protein A, a component of the cell wall of *S. aureus* known to impair opsonization (12, 13, 15), is reduced when *S. aureus* is grown in the presence of low concentrations of clindamycin (3). Therefore, we studied the influence of subinhibitory concentrations of clindamycin on the opsonophagocytosis of *S. aureus* strains with different amounts of protein A.

Bacteria. Opsonophagocytosis of *S. aureus* Cowan I; *S. aureus* Cowan I NG, a protein A-deficient mutant of *S. aureus* Cowan I; *S. aureus* Wood 46; *S. aureus* HSmR; and three strains isolated from patients with staphylococcal infections was studied. The strains were grown overnight at 37°C in Mueller-Hinton broth (MHB; Difco Laboratories, Detroit, Mich.) containing 5 μCi of [³H]thymidine (Amersham International plc, Amersham, United Kingdom) per ml in the presence or absence of one-quarter or one-half of the MIC of clindamycin. For trypsin treatment, 10^{10} radioactively labeled *S. aureus* isolates, grown overnight in MHB without clindamycin, were incubated in 5 ml of trypsin (1 mg/ml; Boehringer GmbH, Mannheim, Federal Republic of Germany) for 30 min at 37°C. A total of 5 ml of trypsin inhibitor (1 mg/ml; from lyophilized soybean; Boehringer) was used to stop the reaction (37°C for 15 min).

Antibiotics. Clindamycin hydrochloride was obtained from The Upjohn Co., Ede, The Netherlands. The MIC of clindamycin was 0.08 μg/ml for *S. aureus* Wood 46 and 0.16 μg/ml for the other *S. aureus* strains tested.

Polymorphonuclear leukocytes. Polymorphonuclear leukocytes (PMNs) were isolated from fresh blood of healthy volunteers. Briefly, after dextran sedimentation of the erythrocytes, PMNs were separated from mononuclear cells by centrifugation on Ficoll-Paque (Pharmacia, Fine Chemicals AB, Uppsala, Sweden). Residual erythrocytes were then lysed by hypotonic NH_4Cl treatment. PMNs were washed and suspended in Hanks balanced salt solution containing 0.1% gelatin (1). Contamination with mononuclear cells

never exceeded 2%. More than 95% of the cells were viable, as shown by trypan blue exclusion.

Serum sources. Normal human pooled serum was obtained from 10 healthy donors and stored at −70°C until use.

Phagocytosis assay. The uptake of radioactively labeled bacteria by PMNs was measured by the method of Verhoef et al. (14). Briefly, radioactively labeled bacteria were opsonized in different concentrations of serum for various periods of time. Equal amounts of preopsonized staphylococci (5×10^7/ml) and PMNs (5×10^6/ml) were incubated in a 37°C shaking water bath. At indicated times phagocytosis was stopped by the addition of ice-cold phosphate-buffered saline. The percentage of staphylococci taken up by the PMNs at each time interval was calculated from the uptake of radioactivity by the phagocytes and the total added radioactivity. Radioactivity was determined by liquid scintillation counting (Philips, Almelo, The Netherlands).

Quantitation of cell wall protein A. Bacteria grown overnight in MHB in the presence or absence of clindamycin were incubated with phosphate-buffered saline containing 2.0 μg of lysostaphin per ml (Schwarz/Mann, Orangeburg, N.Y.) for 120 min at 37°C. The amount of protein A in the lysate was determined quantitatively by indirect hemagglutination with 200 μl of 1% sheep erythrocytes that were previously sensitized with a subagglutinating dose of immunoglobulin G antibody against sheep erythrocytes (National Institute for Public Health, Bilthoven, The Netherlands) (11).

In Fig. 1 it is shown that incubation in subinhibitory concentrations of clindamycin results in enhancement of phagocytosis for bacteria that are rich in protein A but not for bacteria that are protein A deficient, when 1% normal serum is used as the opsonic source. Results of previous studies have shown that the enhanced uptake observed is not due to increased attachment of the clindamycin-treated bacteria to the granulocytes but results from enhanced ingestion by the granulocytes (7, 10).

To further investigate the potential role of protein A in preventing opsonization, strains were treated with trypsin to remove cell surface proteins. Trypsin treatment of the strains grown in the absence of clindamycin resulted in values of protein A (Table 1) and rates of opsonophagocytosis (Fig. 1) that were comparable to those of strains treated with one-half the MIC of clindamycin.

The total amount of protein A present at the bacterial cell surface was determined quantitatively in both clindamycin-
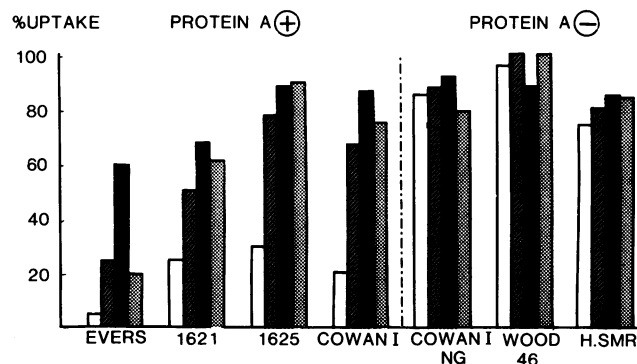
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FIG. 1. Influence of clindamycin (▨, 0.25× MIC; ■, 0.5× MIC) and trypsin (■) on uptake of protein A-rich and protein A-poor *S. aureus* strains (□, control bacteria). Bacteria were opsonized in 1% normal serum for 1 min.

treated and in untreated bacteria. When protein A-rich *S. aureus* strains were grown overnight in the presence of subinhibitory concentrations of clindamycin, the amount of protein A at the bacterial cell surface was significantly reduced (Table 1). In protein A-poor strains, protein A was undetectable in both the control and clindamycin-treated bacteria. According to these findings protein A-poor *S. aureus* strains are so easily opsonized and phagocytized that it is impossible to see any enhancement of opsonophagocytosis after clindamycin treatment when 1% normal serum is used as the opsonic source. Therefore, we diluted the serum to 0.5 and 0.1%. In these low serum concentrations, in which the rates of uptake of the untreated strains were reduced to values beween 50 and 10%, no enhancement of phagocytosis of protein A-poor strains could be observed (data not shown).

The results indicate that the inhibition of protein A synthesis by subinhibitory concentrations of clindamycin in *S. aureus* may be the mechanism for the enhanced uptake of clindamycin-treated *S. aureus* by PMNs. Because clindamycin is considered to be a general inhibitor of protein synthesis, it is reasonable to assume that clindamycin inhibits the synthesis of protein A and other structural proteins and enzymes in *S. aureus*. It is possible that enzymes involved in

TABLE 1. Reduction of the amount of protein A by clindamycin and trypsin in protein A-rich *S. aureus* strain

S. aureus strain	Amt (μg/10⁹ bacteria) of protein A reduction by[a]:			
	Control	0.25× MIC of clindamycin	0.5× MIC of clindamycin	Trypsin
Evers	5	0.16	ND[b]	ND
1621	1.25	0.32	ND	ND
1625	20	0.16	0.16	0.32
Cowan I	20	1.25	0.32	0.64

[a] Minimal detectable amount of protein A in our assay was 0.16 μg/10⁹ bacteria.
[b] ND, Not detectable.

peptidoglycan synthesis and assembly are defective or quantitatively deficient, resulting in peptidoglycan abnormalities. Therefore, studies need to be extended to other surface characteristics that interfere with phagocytosis.

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